TULANE STUDIES IN ZOOLOGY AND BOTANY

Volume 23, Number 2 December 15, 1982

CHANGES IN MELANIN MIGRATION INDUCED BY NORADRENERGIC AND HISTAMINERGIC AGENTS IN THE FIDDLER CRAB, *UCA PUGILATOR**

MUKUND M. HANUMANTE AND MILTON FINGERMAN
Department of Biology, Tulane University
New Orleans, Louisiana 70118 U.S.A.

ABSTRACT

The effects of the H_1 receptor blocker SA-97, the H_2 receptor blocker cimetidine, the tyrosine hydroxylase inhibitor α-methyl-para-tyrosine and the H_1 receptor and norepinephrine uptake$_1$ blocker diphenhydramine on histamine- or 4-methyl histamine-induced inhibition of melanin dispersion in the fiddler crab, *Uca pugilator* undergoing a background transfer from white to black were determined. Only cimetidine significantly antagonized the 4-methyl histamine-evoked decrease in melanin dispersion. α-Methyl-para-tyrosine by itself significantly diminished whereas diphenhydramine by itself significantly potentiated the amount of this centrifugal melanin migration in the fiddler crabs. None of these drugs affected melanin migration *in vitro*. The results are consistent with the hypotheses that norepinephrine triggers release of a melanin-dispersing hormone and that H_2 receptor activation decreases impulse-mediated norepinephrine release in this crab.

INTRODUCTION

Translocation of the melanin in the melanophores of the fiddler crab, *Uca pugilator,* is regulated by antagonistic neurohormones, a melanin-dispersing hormone (MDH) and a melanin-concentrating hormone (Carlson, 1935; Sandeen, 1950; Fingerman, 1956). Norepinephrine (NE) triggers release of MDH in this crab (Fingerman et al., 1981; Hanumante and Fingerman, 1981a,b; 1982a,b,c; Hanumante et al., 1981). Recently histamine (HA) has been shown to inhibit melanin dispersion in a dose-dependent manner (Hanumante and Fingerman, 1981b). Use of a variety of histaminergic agonists and antagonists led to the hypothesis that two types of HA receptors, called H_1 and H_2, are present on NE neurons that trigger MDH release and that HA exerts its inhibitory action by stimulating the H_2 receptors. The present investigation was devised to obtain further support for this hypothesis. This objective was carried out by observing the effects of specific mammalian histaminergic and noradrenergic agents not used previously on the inhibitory action of HA and 4-methyl histamine (4-MeHA; a selective H_2 receptor agonist, Owen et al., 1979; Douglas, 1980; Polanin et al., 1981) on melanin dispersion in *Uca pugilator* transferred from a white to a black background.

MATERIALS AND METHODS

Adult male fiddler crabs, *Uca pugilator,* from the vicinity of Panacea, Florida, (Gulf Specimen Company) were used. Their melanophores were staged according to the system of Hogben and Slome (1931) whereby stage 1.0 represents maximal pigment concentration, stage 5.0 maximal pigment dispersion and stages 2.0, 3.0, and 4.0 the intermediate conditions. When intact crabs were used, the melanophores seen through the cuticle on the anteroventral surface of the second walking leg on the right side were staged at the time a sub-

*Supported by Grant PCM-81-08864 from the National Science Foundation.

stance was injected and 15, 30, 60, 90, and 120 minutes thereafter. To facilitate comparison of the responses of the experimental and control crabs, mean differences between the 15 through 120 minute melanophore stages for the control and experimental groups were calculated for use in Table 1. The depicted data are based on the mean melanophore stages of 20 intact crabs (10 experimental and 10 control) or 20 isolated legs (10 experimental and 10 control). When assays were performed on isolated legs, the melanophores were staged only at the time the legs were removed from the crab (at which time the legs were perfused with the test or control solution) and 15, 30, 45, and 60 minutes thereafter. The second and third walking legs from both sides of the crab were removed; the legs from the right served as experimentals and the legs from the left side received control solution; the melanophores on the anteroventral surface of these isolated legs were observed for staging. The assays were performed using isolated legs having initially either maximally concentrated melanin (stage 1.0) or maximally dispersed melanin (stage 5.0). Melanophores in isolated legs of this crab remain responsive for at least 120 minutes (Herman and Dallmann, 1975). The statistical significance of the data was determined using Standard Errors of the Means (SEM) the Student's *t* test with significance set at the 95% confidence interval. None of the data for isolated legs were statistically significant.

The volume of the solution injected into each crab or isolated leg was always 0.05 ml. The experiments with intact crabs and isolated legs were performed at 24 °C under an illumination of 1190 lx. 4-MeHA dihydrochloride (Smith, Kline and French), cimetidine (N''-Cyano-N-methyl-N'-{2-(5-methylimidazol-4-yl) methylthioethyl} guanidine) (Smith, Kline and French) and SA-97 (homochlorcyclizine) (Eisai) were generous gifts. In addition, HA, α-methyl-para-tyrosine (α-MPT) and diphenhydramine HCl (all from Sigma) were used. The concentration used for each drug, whether injected alone or in combination, was 20 ug/dose of the free compound. All drugs except cimetidine were dissolved in Pantin's physiological saline (Pantin, 1934). Cimetidine was dissolved in acidified (a drop of 1.2 M HCl) saline. Consequently, a drop of HCl (1.2 M) was added to control saline for the cimetidine experiments. The rest of the controls received pure saline.

Results and Discussion

4-MeHA, an H_2 receptor agonist, slowed the rate of melanin dispersion, as observed earlier by Hanumante and Fingerman (1981b), in intact crabs transferred from a white to a black background (Table 1). Cimetidine, which selectively blocks mammalian H_2 receptors (Douglas, 1980; Polanin and McNeill, 1981) significantly antagonized the 4-MeHA. On the other hand, the H_1 receptor blocker SA-97 not only did not antagonize the 4-MeHA but the combination of 4-MeHA plus SA-97 resulted in significantly further inhibition. None of these drugs affect melanin migration *in vitro* nor do SA-97 and cimetidine by themselves have an effect on the rate of melanin dispersion in crabs undergoing a background change from white to black (Hanumante and Fingerman, 1981b), a black background fostering melanin dispersion (Brown and Hines, 1952) which will be effected by MDH.

α-MPT selectively inhibits tyrosine hydroxylase. This enzyme catalyzes the synthesis of dihydroxyphenylalanine from tyrosine. At least in mammals this is the rate-limiting step in the biosynthesis of NE (Terrasawa et al., 1975; Lofström and Backström, 1978). α-MPT by itself significantly decreased melanin dispersion. HA by itself, as reported earlier (Hanumante and Fingerman, 1981b), significantly reduced centrifugal melanin migration in intact crabs transferred from a white to a black background. However, in the crabs that were co-administered either 4-MeHA and α-MPT or HA and α-MPT (Table 1), 4-MeHA and HA were not able to produce further, significant

reduction of the melanin dispersion. Diphenhydramine, a blocker of H_1 receptors and NE uptake$_1$ in mammals (Isaac and Goth, 1965; Fantozzi et al., 1975; Marco et al., 1980), by itself significantly enhanced melanin dispersion. However, when HA was co-administered with diphenhydramine, the HA-induced inhibition in melanin dispersion was still evident (Fig. 1).

The present data, in light of our earlier report (Hanumante and Fingerman, 1981b) and the pharmacological actions of noradrenergic and histaminergic agents in mammals, further strengthen the hypothesis that (a) NE serves as a neurotransmitter triggering release of MDH and that (b) activation of H_2 receptors located on NE neurons which control MDH release results in a decrement of melanin dispersion in *Uca pugilator* transferred from a white to a black background. The observations that cimetidine, a selective H_2 receptor blocker, antagonized the 4-MeHA-induced inhibition in melanin dispersion, whereas the H_1 blocker SA-97 did not, reveal that this effect is mediated specifically by activation of HA H_2 receptors. The marked increase in inhibitory effect of 4-MeHA when co-administered with the H_1 antagonist SA-97 was probably due to the fact that excitation of H_1 receptors evokes enhanced melanin dispersion (Hanumante and Fingerman, 1981b), blocking them would prevent any endogenous H_1 stimulation of the crabs. This would enable 4-MeHA, an agonist of H_2 receptors, to produce an even greater inhibition of the melanin dispersion. On the contrary, in the crabs whose H_2 receptors were blocked by cimetidine, 4-MeHA was unable to significantly decrease the action potential-mediated release of NE, which in turn resulted in a near normal quantity of MDH being released into the hemolymph of these crabs transferred to the black background. The fact that metiamide, another H_2 receptor blocker, significantly antagonized the 4-MeHA-stimulated decrease in centrifugal melanin migration (Hanumante and Fingerman, 1981b) *in vivo* further strengthens this conclusion.

NE has been found (0.51 μg/g) in the supraesophageal ganglia of male fiddler crabs (Hanumante and Fingerman, 1982b). Also, we have provided evidence that H_1 and H_2 receptors occur on NE neurons because in fiddler crabs pretreated with 6-hydroxydopamine (which presumably destroys NE neuroterminals in *Uca* as it does in vertebrates) (Hanumante and Fingerman, 1982b,c) HA is unable to significantly reduce further the melanin dispersion (Hanumante and Fingerman 1981b). We have not determined (i) the levels of NE in α-MPT injected crabs or (ii) the exact mechanism of action of α-MPT in *Uca puilator*. However, data that we obtained using noradrenergic and histadrenergic agents (Hanuamante and Fingerman, 1981b) reveal that 20 MPT clearly interferes with NE neurotransmission. This probably was either by way of its well-established (at least in mammals) pharmacological NE synthesis-inhibiting effect (Terraswawa et al., 1975; Lofström and

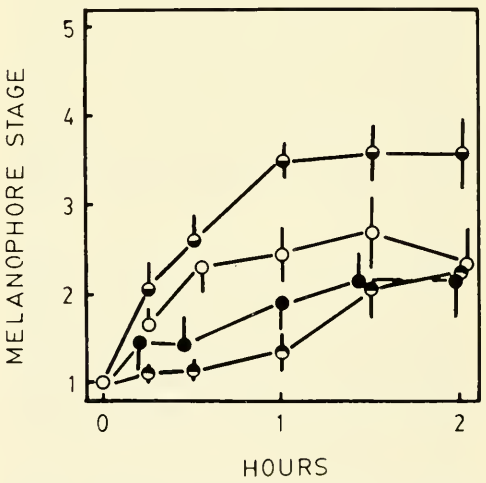

Figure 1. Relationships between melanophore stage and time. Circles with bottom-half darkened, crabs that received diphenhydramine; circles with top-half darkened, crabs that received histamine; solid circles, crabs that received histamine plus diphenhydramine; open circles, saline-injected controls. Vertical bars indicate SEM.

TABLE 1. The means (± SEM) of the differences between the melanophore stages determined at 15, 30, 60, 90, and 120 minutes of the intact crabs that received a drug versus the saline-injected controls. The minus sign indicates decreased melanin dispersion relative to the controls. *Statistically significant $p \leq .05$ relative to respective controls.

4-Methyl histamine (4-MeHA)	−0.67* (± 0.08)
Cimetidine	−0.17 (± 0.01)
4-MeHA plus cimetidine	−0.39 (± 0.07)
4-MeHA plus SA-97	−1.43* (± 0.12)
α-Methyl-p-Tyrosine (α-MPT)	−1.15* (± 0.15)
4-MeHA plus α-MPT	−1.44* (± 0.21)
Histamine (HA)	−1.18* (± 0.18)
HA plus α-MPT	−1.01* (± 0.12)

Backström, 1978; Douglas, 1980) or by stimulating H_2 receptors, thereby leading to the observed decrement in MDH release (Table 1). Hence, the melanin of these α-MPT-treated crabs did not disperse to the extent it did in the control animals.

As stated above, in the crabs co-injected with 4-MeHA and α-MPT or HA and α-MPT, neither 4-MeHA nor HA significantly affected the melanin dispersion compared with that which occurred in response to α-MPT alone (Table 1). This presumably was due to the interference with NE neurons by α-MPT in such a way that the impulse-mediated decrement in NE secretion by the H_2 stimulators 4-MeHA and HA was not large enough to affect significantly the NE-mediated MDH release.

The diphenhydramine-evoked increment in melanin dispersion (Fig. 1) was presumably due to its blocking action on NE uptake$_1$ (Marco et al., 1980). NE uptake$_1$ inhibitors like nisoxetine (Koe, 1976) have already been shown to potentiate MDH release (Hanumante and Fingerman, 1981a). Diphenhydramine antagonizes H_1 receptors (Isaac and Goth, 1965; Fantozzi et al., 1975; Marco et al., 1980) also. However, because H_1 receptor blockers do not significantly abolish HA- or 4-MeHA- (an H_2 receptor agonist) mediated inhibition of melanin dispersion, we suggest that the NE uptake$_1$ blocking action of diphenhydramine is responsible for the potentiation of melanin dispersion. The observation that even when HA is co-administered with diphenhydramine there is still a decrease in melanin dispersion (Fig. 1) indicates that HA does not evoke its effect by stimulating NE uptake$_1$; uptake$_1$ being the major mechanism of inactivating the postsynaptic actions of monoamines including NE (Fuller and Wong, 1977). That none of these drugs affect significantly melanin migration in isolated legs (Hanumante and Fingerman, 1981b) is consistent with the hypothesis that these drugs elicit changes in melanin dispersion indirectly by interacting with the neuroendocrine system of *Uca pugilator*.

LITERATURE CITED

BROWN, F.A., JR., and M.N. HINES. 1952. Modifications in diurnal pigmentary rhythm of *Uca* affected by continuous illumination. Physiol. Zool. 25: 56-70.

CARLSON, S.P. 1935. The color changes in *Uca pugilator*. Proc. Nat. Acad. Sci. 28: 549-551.

DOUGLAS, W.W 1980. Histamine and 5-hydroxytryptamine (serotonin) and their antagonists, pp. 609-646 *In:* A.G. Gilman et al., Eds. Goodman and Gilman's The pharmacological basis of therapeutics, 6th ed., Macmillan Publishing Co., New York.

FANTOZZI, R., F. FRANCONI, P.E. MANNAIONI, E. MASINI, F. MORONI. 1975. Interaction of H_1- and H_2- receptor antagonists with histamine uptake and metabolism by guinea-pig isolated atrium and mouse neoplastic mast cells

in vitro. Br. J. Pharmacol. 53: 569-574.

FINGERMAN, M. 1956. Black pigment concentrating factor in the fiddler crab. Science 123: 585-586.

_____, M.M. HANUMANTE, S.W. FINGERMAN, and D.C. REINSCHMIDT. 1981. Effects of norepinephrine and norepinephrine agonists and antagonists on the melanophores of the fiddler crab, *Uca pugilator.* J. Crust. Biol. 1: 16-26.

FULLER, R.W., and D.T. WONG. 1977. Inhibition of serotonin reuptake. Fed. Proc. 36: 2154-2158.

HANUMANTE, M.M., and M. FINGERMAN. 1981a. Responses of the melanophores of the fiddler crab, *Uca pugilator,* to drugs affecting noradrenergic neurotransmission: Further evidence for norepinephrine as a neurotransmitter triggering release of melanin-dispersing hormone. Comp. Biochem. Physiol. 70C: 27-34.

_____. 1981b. Inhibitory effect of histamine on the release of melanin-dispersing hormone in the fiddler crab, *Uca pugilator.* Amer. Zool. 21: 1011.

_____. 1982a. Additional evidence for norepinephrine as a neurotransmitter triggering release of melanin-dispersing hormone in the fiddler crab, *Uca pugilator:* The effects of alpha$_1$ and beta adrenoceptor blocking drugs on melanin migration. Comp. Biochem. Physiol. 71C: 15-19.

_____. 1982b. Further evidence for norepinephrine as a neurotransmitter stimulating release of melanin-dispersing hormone in the fiddler crab, *Uca pugilator:* The changes in the melanophores of the crabs following reserpine, 6-hydroxydopamine and bretylium administration. Gen. Pharmacol. 13: 99-103.

_____. 1982c. Pharmacological involvement of presynaptic alpha$_2$ adrenoceptors in norepinephrinergic neurotransmission triggering the release of melanin-dispersing hormone in the fiddler crab, *Uca pugilator.* J. Crust. Biol. 2: 22-30.

_____, S.W. FINGERMAN, and M. FINGERMAN. 1981. Antagonism of the inhibitory effect of the polychlorinated biphenyl preparation, Aroclor 1242, on color changes of the fiddler crab, *Uca pugilator,* by norepinephrine and drugs affecting noradrenergic neurotransmission. Bull. Environ. Contamin. Toxicol. 26: 479-484.

HERMAN, W.S., and S.H. DALLMANN. 1975. *Limulus* chromatophorotropin: action on isolated *Uca* legs and in various crustaceans. Experientia 31: 918-919.

HOGBEN, L., and D. SLOME. 1931. The pigmentary effector system - VI. The dual character of endocrine co-ordination in amphibian colour change. Proc. R. Soc. Lond. B. 108: 10-53.

ISAAC, L., and A. GOTH. 1965. Interaction of antihistaminics with norepinephrine uptake: cocaine-like effect. Life Sci. 4: 1899-1904.

KOE, K. 1976. Molecular geometry of the inhibitors of the uptake of catecholamines and serotonin in synaptosome preparations of rat brain. J. Pharmac. Exp. Ther. 199: 649-661.

LOFSTRÖM, A., and T. BACKSTRÖM. 1978. Relationship between plasma estradiol and brain catecholamine content in the diestrus female cat. Psychoneuroendocrinology 3: 103-107.

MARCO, E.J., G. BALFAGON, J. MARIN, B. GOMEZ, and S. LLUCH. 1980. Indirect adrenergic effect of histamine in cat cerebral arteries. Naunyn-Schmiedeberg's Arch. Pharmacol. 312: 239-243.

OWEN, D.A.A., C.A. HARVEY, and R.W. GRESTWOOD. 1979. Cardiovascular studies with impromidine (S. K. and F. 92676), a new very potent and specific histamine H_2-receptor agonist. J. Pharm. Pharmacol. 31: 577-582.

PANTIN, C.P.A. 1934. The excitation of crustacean muscle. J. Exp. Biol. 11: 11-27.

POLANIN, A., and J.H. MCNEILL. 1981. Characterization of the histamine receptors in rabbit left atria. Can. J. Physiol. Pharmacol. 59: 19-24.

_____, T.E. TENNER, JR., and J.H. MCNEILL. 1981. The characterization of cardiac histaminergic chronotropic receptors in the rabbit. Can. J. Physiol. Pharmacol. 59: 14-18.

SANDEEN, M.I. 1950. Chromatophorotropins in the central nervous system of *Uca pugilator,* with special reference to their origins and actions. Physiol. Zool. 23: 337-352.

TERRASAWA, E., W.E. BRIDSON, J.W. DAVENPORT, and R.W. GAY. 1975. Role of brain monoamines in release of gonadotropin before proestrus in the cyclic rat. Neuroendocrinology 18: 345-359.